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Effect of Simulated Acid Rain on Nitrification and Nitrogen Mineralization in Forest Soils

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16. ABSTRACT

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Effect of Simulated Acid Rain on Nitrification and Nitrogen Mineralization in Forest Soils[1]

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ABSTRACT

To determine the possible microbiological changes in soil resulting from acid rain, columns containing samples of forest soils were leached with either a continuous application of 100 cm of simulated acid rain (pH 3.2–4.1) at 5 cm/hour or an intermittent 1.5-hour application of 1.2 cm of simulated acid rain twice weekly for 19 weeks. The upper 1.0- to 1.5-cm portions of soil from treated columns were used to determine the changes in inorganic N levels in the soil. Nitrification of added ammonium (NH_4^+) was inhibited following continuous exposure of soil to simulated acid rain of pH 4.1–3.2. The extent of the inhibition was directly related to the acidity of the simulated rain solutions. The production of inorganic N in the absence of added NH_4^+ was either stimulated or unaffected following continuous treatment of soils with pH 3.2 simulated acid rain. The addition of nitrapyrin (2-chloro-6-[trichloromethyl]pyridine), an inhibitor of autotrophic nitrification, caused a decrease in nitrification in water-treated soil but had little effect on nitrification in soil treated with pH 3.2 simulated acid rain. Intermittent applications of simulated acid rain (pH 3.5–4.1) for 19 weeks partially inhibited nitrate (NO_3^-) production in soil amended with NH_4^+ following the exposure period, but NO_3^- production in unamended soil was either unaffected or stimulated.

Additional Index Words: air pollution, heterotrophic nitrification.

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An environmental stress affecting a step in some portion of the N cycle could have serious ecological consequences because N transformations are often critical for plant growth. Acid precipitation, which affects large areas of the northeastern United States and northern Europe, is potentially one of those stresses, but little is known about the effects of acid precipitation on the microbial components of terrestrial ecosystems. Most of the studies concerning acid precipitation have focused on its effects on aquatic ecosystems (Wright et al., 1976; Hendrey et al., 1976) or on the macrobiota or chemical changes in terrestrial ecosystems (Dochinger and Seliga, 1976). A few investigations have dealt with microbial processes in soil (Baath et al., 1979; Tamm et al., 1977).

Changes in soil pH associated with agricultural practices are known to influence individual N transformations; for example, the liming of acid soils has been shown to stimulate nitrification (Dancer et al., 1973; Nyborg and Hoyt, 1978) and N-mineralization (Ishaque and Cornfield, 1972), although neither Dancer et al. nor Nyborg and Hoyt were able to show that N-mineralization was greatly affected by soil pH. Dry deposition, which includes absorption of SO_2 and NO_x by soil and other surfaces, and which is one form of acid precipitation, has been shown by Labeda and Alexander (1978) to inhibit nitrification in certain soils, although whether the inhibition was caused by the solubility products of SO_2 or a lowering of soil acidity was not known.

The present investigation was undertaken to determine if nitrification and N-mineralization are susceptible to acid precipitation. Although the acid concentrations used were higher than ambient levels and the durations of rain applications were short, the results obtained should show which processes and soils are not affected and which require further study. The investigation is part of an integrated study of the effect of acid rain on watersheds of the Adirondacks region of New York.

MATERIALS AND METHODS

The soils used were Potsdam-Crary gravelly loamy sand (frigid typic or aquic fragiorthod, pH 4.4), collected from the Panther Lake catchment basin in the Adirondacks Mountains of New York, and two samples of Hudson silt loam (glossaquic hapludalf), one at pH 5.6 and one at pH 6.3. Rectangular cores, 10–20 cm deep, were taken and transported to the laboratory, and a section of the core was passed through a 4-mm sieve immediately prior to being placed in either a 3.5- or 10-cm diam plastic cylinder. The smaller cylinders were used in the early part of the study, but they did not contain an adequate amount of soil for later experiments. The sieved soil was retained in the cylinder by a fiberglass screen and a Whatman GF/A glass fiber filter. The method of exposure of the soil columns to artificial rain has already been described (Strayer and Alexander, 1981), except that the rain was supplied through four evenly spaced capillary pipettes for the 10-cm diam. column instead of the one pipette used for the smaller column. The rain application rate was 0.8 and 5.0 cm/hour for the 10-

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and 3.5-cm diam. columns, respectively. Stock solutions of pH 4.1 simulated acid rain were formulated from published reports by Cogbill and Likens (1974) and Evans and Raynor (1976), and the composition has been previously described (Strayer and Alexander, 1981). When necessary, stock solutions of simulated acid rain were adjusted to lower pH values with HCl. The soil columns were exposed to: (i) continuous rain application equivalent to 100 cm of rain for 1–2 days and (ii) intermittent applications equivalent to 1.2 cm of rain twice a week for 19 weeks to assess the effects of chronic episodes of acid rain.

After the columns were exposed to the synthetic rain, the soil in the surface 1.0–1.5 cm was removed, and a composite was made by pooling sections from replicate columns. Only the topmost portion was sampled because the greatest effects were found to occur near the surface (Strayer and Alexander, 1981). These composites were dried to a moisture content that was just below 70% of field capacity, and portions were placed in 50- or 125-ml Erlenmeyer flasks. The flasks were closed with styrofoam plugs, and distilled, deionized water was added at 3- to 5-day intervals to maintain the moisture content. Amendments were made to the soil in the flasks, and the soil moisture content was brought up to 70% of field capacity. In some experiments, nitrapyrin (2-chloro-6-[trichloromethyl]pyridine; Dow Chemical Co., Midland, Mich.), a specific inhibitor of autotrophic nitrification (Shattuck and Alexander, 1963), was added to the amendment solution in a minimal volume of methanol to give a soil concentration of 10 μg/g of dry soil. The temperature of incubation was 20–22°C.

Ammonium was extracted from soil with the 2.0*N* KCl-extracting solution of Bremner (1965). Soil samples (1.0 g) were shaken for 1 hour with 10 ml of the extracting solution and 0.1 g Darco G-60 (Atlas Chemical Co., Wilmington, Del.), and then the mixture was filtered through Whatman no. 40 filter paper. The filtrates were analyzed for NH_4^+ by the phenate method of Weatherburn (1967). Soil NO_3^- and NO_2^- were extracted with a 0.2% $Ca(OH)_2$ solution as described by Labeda and Alexander (1978). The extracts were analyzed for NO_3^- by the modified Griess-Ilosvay method of Downey (1966) and for NO_2^- by the chromotropic acid procedure (APHA, 1971). A water-to-soil ratio of 1:1 (v/w) was used for measuring soil pH as described by Peech (1965).

RESULTS

Columns of Hudson silt loam (initial pH of 5.6) were exposed to treatments of 100 cm of either distilled, deionized water or pH 3.2 simulated acid rain. After exposure, the composite soil samples obtained from the top 1.5 cm of the columns were amended with the 100 μg of NH_4^+-N/g (as the SO_4^{2-} salt), and the production of NO_3^- was periodically measured for the next 28 days (Fig. 1). In the water-treated soil, which had a pH of 5.6 after treatment, NO_3^- levels increased with time. In the soil treated with simulated acid rain, the pH of the soil falling to 4.5 after treatment, little NO_3^- was produced. Similar results were obtained with a sample of Hudson silt loam that had a pH of 6.3 prior to treatment with the synthetic acid rain.

The pH of the simulated rain was adjusted to values ranging from 3.3 to 4.1 to determine the pH of rain that completely inhibited nitrification. Following exposure of the Hudson soil (initial pH of 5.6), the net changes in NO_3^- and NH_4^+ in soils were measured at the end of a 16-day incubation period. This time was selected because about half of the NH_4^+ added to the water-treated soil shown in Fig. 1 had been converted to NO_3^- in this time. The results show that increasing acidity of the simulated rain caused a decrease in soil pH and in the net change of NO_3^- and NH_4^+ (Table 1). Even the mildest simulated rain treatment of pH 4.12 caused a decrease in NO_3^- formation and NH_4^+ disappearance, showing the marked sensitivity of the responsible populations. Nitrification was abolished in the soil that had been treated with pH 3.55 simulated acid rain. The net change in NO_3^- levels shown in Table 1 was calculated by subtracting the amount of NO_3^- produced in the soil not amended with NH_4^+ from the amount of NO_3^- produced in the NH_4^+-amended soil. In the soils not receiving NH_4^+, NO_3^- production in the samples treated with simulated acid rain (18 μg NO_3^--N/g of soil) was not appreciably different from that in the water-treated soils (15 μg/g of soil).

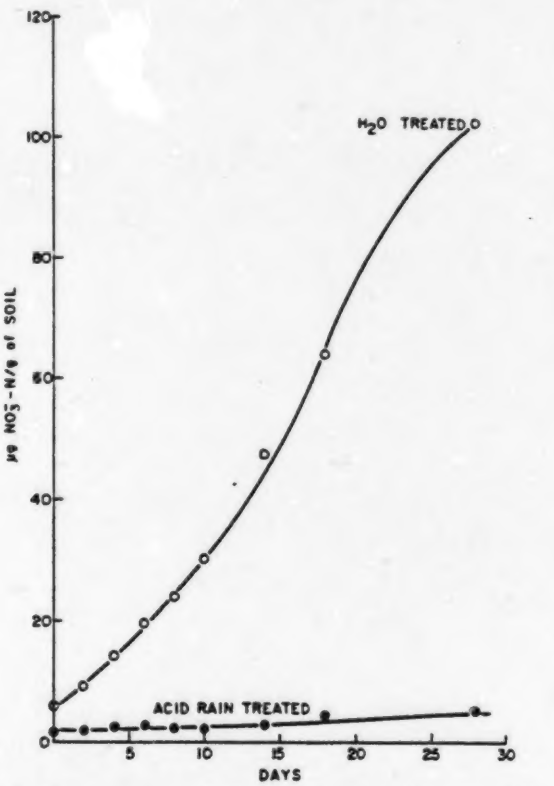

Fig. 1—Nitrate production in Hudson silt loam previously exposed to a continuous application of either distilled, deionized water or pH 3.2 simulated acid rain.

Because acid rain did not adversely influence the activity in soil not receiving NH_4^+, the effects of simulated acid rain were investigated further by monitoring the in-

Table 1—Effect of the continuous application of simulated rain with different pH values on nitrification in Hudson silt loam (pH 5.6) amended with 100 μg NH_4^+-N/g following exposure and incubated for 16 days.

Treatment pH	Soil pH	μg N change/g of soil	
		NO_3^-	NH_4^+
5.75†	5.67	44 ± 1.3‡	−52 ± 16
4.12	5.42	30 ± 0.6	−39 ± 8.2
3.83	5.19	20 ± 3.6	−12 ± 4.0
3.55	4.88	−0.5 ± 1.3	−0.4 ± 7.9
3.31	4.42	−3.0 ± 0.3	−6.3 ± 5.2

† Treated with distilled, deionized water.
‡ Mean of three replicates ± standard error. A positive value denotes a net increase, and a negative value a net decrease, in the ion concentration.

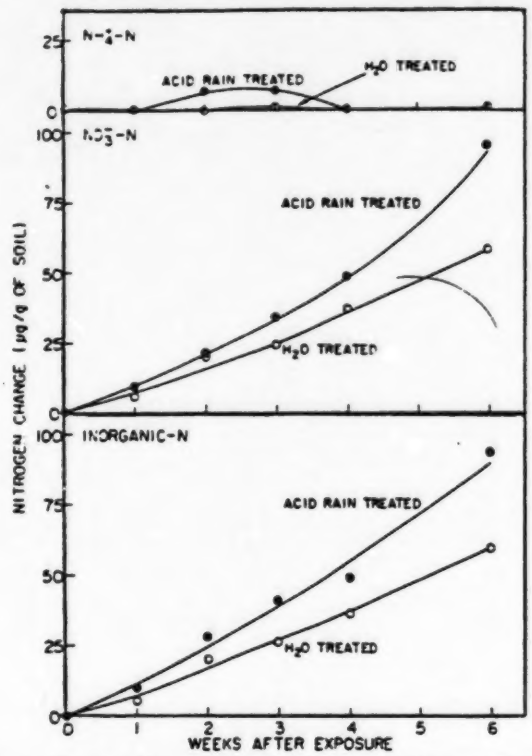

Fig. 2—Nitrogen mineralization (as net change in inorganic-, NO_3^--, and NH_4^+-N) in Hudson silt loam (pH 5.6) treated with water or pH 3.2 simulated acid rain. Inorganic N is the sum of NH_4^+-N, nitrite-N, and NO_3^--N.

organic N levels in unamended Hudson silt loam (pH 5.6) for 6 weeks following exposure to rain. The results showed that treatment with pH 3.2 simulated acid rain increased the production of inorganic N, mostly as NO_3^-, when compared with inorganic N levels in water-treated soil (Fig. 2). A temporary accumulation of NH_4^+ was also observed in the soil treated with pH 3.2 simulated acid rain. Thus, in the one soil tested, simulated acid rain inhibited NO_3^- production in NH_4^+-amended soil but had the opposite effect in unamended soil.

A study was also conducted of the effects of exposures to 100 cm of simulated acid rain on nitrification and N mineralization in Potsdam-Crary soil (pH 4.4). In previous experiments, the acid rain solutions contained NH_4^+ (22 μeq/liter) and NO_3^- (45 μeq/liter), whereas the distilled, deionized water treatments did not. Because the addition of inorganic N to the soil by the simulated acid rain would alter the NO_3^- and NH_4^+ levels and thus possibly influence N transformations, the Potsdam-Crary soil was subjected to a third rain solution for comparison. This solution was identical in composition with the pH 3.2 simulated acid rain except that the H^+ ions were replaced by K^+ ions. Therefore, soil treated with this solution would receive the same quantities of NH_4^+ and NO_3^- as soil receiving the simulated acid rain; such a control for inorganic N was lacking in the treatments with distilled water. Nitrapyrin was added to some samples of both NH_4^+-amended and unamended soils after the exposure period in order to inhibit autotrophic nitrification.

The net amounts of NO_3^- produced were similar after soil was exposed to simulated acid rain, K^+ rain, and distilled water and then amended with a solution that did not contain NH_4^+ or nitrapyrin (Table 2), but the net change in NH_4^+ was slightly greater in the K^+-rain-treated soil than in either the water or acid-rain-treated soil. When the soil was amended with a solution that contained nitrapyrin but no NH_4^+, NO_3^- production was inhibited and NH_4^+ accumulated in the water-treated, soils, but not in the simulated acid-rain-treated soil (Table 2). These results suggest either that N was oxidized by heterotrophic organisms in soil exposed to acid rain and by autotrophic microorganisms in soils treated with water or that nitrapyrin is ineffective in the soil receiving acid rain. Because the inhibition was only partial in the soil receiving K^+ rain, nitrification in this soil sample may be a result of both autotrophic and heterotrophic microorganisms.

Amendment of the exposed soils with only NH_4^+ resulted in a slight stimulation of NO_3^- production with a concomitant decrease in NH_4^+ levels in the water- and K^+-rain-treated soil, but NH_4^+ addition did not promote NO_3^- production in the synthetic acid-rain-treated soil (Table 2). The failure of NH_4^+ to stimulate NO_3^- production in the acid-rain-treated soil suggests that the enrichment for autotrophic nitrifiers was inhibited by the acid rain treatment. When nitrapyrin was added to soils that had been exposed to simulated rain treatments and then amended with NH_4^+, the increase in NO_3^- and the decrease in NH_4^+ levels were unaffected in the soils treated with water and simulated acid rain.

Table 2—The effects of continuous exposure to 100 cm of simulated acid rain, K^+ rain, and distilled, deionized water on net changes in NO_3^- and NH_4^+ in Potsdam-Crary soil (pH 4.4) with and without NH_4^+ (100 μg N/g) and nitrapyrin additions (10 μg/g) following exposure.

Treatment	Soil pH	No NH_4^+ added		NH_4^+ added	
		− Nitrapyrin	+ Nitrapyrin	− Nitrapyrin	+ Nitrapyrin
		μg NO_3^--N change per g of dry soil/21 days			
H_2O, pH 5.6	4.27	13 ± 1.1†	−3.7 ± 0.7	21 ± 1.2	24 ± 1.4
K^+ rain, pH 4.4	4.51	12 ± 1.0	4.5 ± 1.7	21 ± 1.2	5.2 ± 1.0
Acid rain, pH 3.2	3.78	11 ± 1.0	10.5 ± 1.3	8.5 ± 1.8	6.8 ± 1.7
		μg NH_4^+-N change per g of dry soil/28 days			
H_2O, pH 5.6	4.27	1.5 ± 0.4	24 ± 0.4	−15 ± 2.5	−12 ± 1.7
K^+ rain, pH 4.4	4.51	4.5 ± 1.0	19 ± 0.5	−18 ± 3.1	11 ± 3.3
Acid rain, pH 3.2	3.78	0.8 ± 1.6	−1.3 ± 0.8	6.2 ± 5.5	6.4 ± 4.2

† Mean of five replications ± standard error. A positive value denotes a net increase, and a negative value a net decrease, in the ion concentration.

The effect of chronic exposures to acid rain was studied by treating the Hudson silt loam (pH 5.6) with intermittent applications of simulated rain for 19 weeks. Soil columns received two applications per week of 1.2 cm of either distilled, deionized water or pH 4.1, 3.8, or 3.5 simulated acid rain. Following the last exposure to simulated rain, the upper 1.5 cm of soil in each column was sampled, solutions containing the amendments were added, and the net amounts of NO_3^- produced in the next 17 days were measured. The amendments included: (i) distilled, deionized water, (ii) NH_4^+ (100 μg N/g of dry soil), (iii) nitrapyrin (10 μg/g of dry soil), or (iv) NH_4^+ plus nitrapyrin in distilled water. The results show that NO_3^- production in only the NH_4^+-amended soils was decreased by the simulated acid rain treatment (Table 3). Exposure of soil to pH 4.1 or 3.5 simulated acid rain caused a slight stimulation in NO_3^- production in exposed soils that had received a distilled water amendment. Nitrate production in either NH_4^+-amended or water-amended soils that also received nitrapyrin was unaffected by chronic episodes of simulated acid rain. Comparisons among soils receiving the different amendments indicated that nitrapyrin increased NO_3^- production in soils not amended with NH_4^+, but significantly decreased NO_3^- production in NH_4^+-amended soil.

DISCUSSION

Nitrification is known to occur in acid soils of both agricultural (Dancer et al., 1973; Ishaque and Cornfield, 1972) and nonfarmed (Smith et al., 1968; Pfadenhauer, 1979) ecosystems, but the rates are usually low when compared with rates in soils of higher pH values. In the present study, soils with different initial pH values were acidified with simulated acid rain to determine if this environmental stress affected nitrification. In the exposed soils amended with NH_4^+ and incubated, simulated acid rain caused a striking decrease in the rate of NO_3^- production in all three soils. However, when exposed soils were not amended with NH_4^+, a condition that more closely approximates natural NH_4^+ levels in a forest soil, simulated acid rain had no detectable adverse influence on the rate of NO_3^- production. The difference in effect between the NH_4^+-amended and unamended soils may be a result of the process being heterotrophic and unaffected by acid rain in the latter instance, but autotrophic following NH_4^+ additions and sensitive to acid rain. Focht and Verstraete (1977) cited unpublished results of Verstraete and Voets that showed that 100 μg NH_4^+-N/g of soil could completely inhibit heterotrophic nitrification in an acid soil. Ishaque and Cornfield (1972) speculated that nitrification in acid Pakistani soils may be heterotrophic.

Nitrapyrin, a compound that inhibits autotrophic but not heterotrophic nitrification (Shattuck and Alexander, 1963), was added to rain-treated soils to determine if the pathway of N oxidation was autotrophic or heterotrophic. The results suggest that nitrification was autotrophic in water-treated Potsdam-Crary soil receiving no NH_4^+ and probably heterotrophic in the simulated acid-rain-treated soil; thus, acid rain treatment may cause a shift in the organisms involved in N oxidation. In the Hudson silt loam receiving no NH_4^+, NO_3^- production was not inhibited by nitrapyrin in either the simulated acid-rain- or water-treated soil, suggesting that nitrification was brought about by heterotrophic microorganisms.

The addition of nitrapyrin to soils enriched with NH_4^+ led to conflicting results. Nitrapyrin partially inhibited NO_3^- production in Hudson silt loam soil exposed to water and simulated acid rain treatments, and nitrapyrin either partially inhibited or did not suppress NO_3^- production in Potsdam-Crary soil receiving NH_4^+., Hendrickson and Keeney (1979) reported that the effectiveness of nitrapyrin as an inhibitor of autotrophic nitrification decreased with decreasing soil pH or with increasing levels of soil organic matter, but the nitrapyrin levels they used were one-10th of the levels used in this study. However, the results obtained with amended soils that also received nitrapyrin cannot be explained by the ineffectiveness of nitrapyrin because the chemical at least partially inhibited NO_3^- production in all but one of the NH_4^+-amended soils, and the one exception had the highest pH.

In agricultural soils, the addition of high levels of NH_4^+ is routine, and the potential inhibition of autotrophic nitrification by acid rain could have important consequences. In forest soils, in contrast, NH_4^+ levels may not reach the concentrations added in this study; thus, the results obtained with the unamended soils suggesting that short-term exposures to acid rain do not inhibit NO_3^- production are more realistic for forest ecosystems, at least when they are exposed for short periods of time. Because nitrification in amended soils was vulnerable to simulated acid rain inputs, with a partial inhibition even by the pH 4.1 rain, this process can be used as a sensitive indicator to forewarn that acid rain may soon be affecting other, less sensitive processes.

Table 3—The effects of 19-week intermittent applications of simulated rain solutions of different pH values on the net production of NO_3^- in Hudson silt loam with and without NH_4^+ and nitrapyrin additions following exposure.

Treatment	Final soil pH	No NH_4^+ added		NH_4^+ added	
		− Nitrapyrin	+ Nitrapyrin	− Nitrapyrin	+ Nitrapyrin
		μg NO_3^--N change/g of dry soil			
H_2O, pH 5.6	5.39	13 ± 0.2†	17 ± 0.7	90 ± 2.2	41 ± 0.6
Acid rain					
pH 4.1	5.29	16 ± 0.4	18 ± 0.4	81 ± 1.0	41 ± 1.1
pH 3.8	5.15	12 ± 1.1	17 ± 2.1	71 ± 1.3	41 ± 1.1
pH 3.5	5.03	17 ± 1.4	17 ± 1.1	68 ± 2.2	38 ± 5.8

† Mean of three replicates ± standard error.

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